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PROBLEMS IN STERILIZATION OF MANNED SPACE VEHICLES

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ABSTRACT

The probability of achieving and maintaining sterility of an unmanned spacecraft with various suggested procedures is examined in detail, as are alternative techniques for avoiding biological contamination of the planets. The required degree of assurance against contamination of Mars, Venus, and the Moon with Earth organisms is also considered. For Mars landers and orbiters, sterilization of the spacecraft or capsule by dry heat, with no subsequent access, is found to be desirable. For the present, sterilization of Venus landers still seems desirable. For Mars flybys, and for Venus orbiters and flybys, control of the trajectory to hold down the chance of unintentionally entering the planetary atmosphere appears the method of choice. For the Moon, sterilization appears unnecessary, but microbial counts should be kept low. Sterilization lowers spacecraft and system reliability. It reduces the chance of launching within periods fixed by astronomical constraints, and increases costs. The gain which should be achieved through spacecraft sterilization, in return of significant biological information about the planets, must be balanced against these losses. In particular, there should be kept in mind the loss in return of biological data occasioned by failure of a spacecraft to fulfill its mission; the probability of such failure is increased by sterilization.

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There is general agreement, at least among biologists, that, to avoid interference with exobiological observations, terrestrial organisms should not be freely released on other planets. Sterilization and other techniques for avoiding man-made biological contamination of the planets may therefore be prerequisite for exobiological research. Considerable controversy has occurred on the need for sterilization of planetary and lunar spacecraft, on the degree of assurance required that contamination does not occur, and on the methods to be used for sterilization. Many extreme and opposing views have been put forth by individuals concerned with or affected by sterilization. This paper attempts an engineering examination of the problem. It deals with unmanned exploration; sterilization of manned spacecraft require separate consideration. Also, only contamination of another planet with Earth organisms is discussed; contamination of Earth with organisms from other planets, when samples or spacecraft are brought back to Earth, is not considered here.

Some persons, on first being faced with the problem of spacecraft sterilization, have felt that it should be a simple matter. After all, surgical instruments and supplies, drugs, food, and other items are sterilized routinely. A more detailed consideration shows, however, that the problem is not simple. Spacecraft components and materials are in many cases damaged by common sterilization methods. Techniques and operations useful for supplies or for small easily assembled devices may be very difficult to apply to a large complex

device, parts of which are likely to be repaired or replaced. Perhaps most important is that careful attention must be paid to the probability of successfully achieving and maintaining sterilization with a given technique; many of the procedures proposed for spacecraft sterilization are poor in this respect.

Sterility is absolute: an object is either sterile or it is not sterile. If it has a single viable organism on it or in it, it is not sterile. Nevertheless, we never really know whether a particular spacecraft is sterile or not. We never really know whether a piece of laboratory equipment or surgical instrument is sterile or not. All we can say is, that on the basis of the procedures used and of past experience, the chance that the object is contaminated is less than some low number. It is, therefore, proper to consider the probability of contamination or the probability of sterility; such an approach will be used in this paper.

In many cases, the probability of sterility resulting from an operation can only be estimated. Nevertheless, it is necessary to proceed on this basis or not proceed at all; any reasonable estimate is better than nothing. As in many engineering problems, engineering judgement must often be resorted to in the absence of conclusive experimental data.

PROBABILITY OF STERILITY ATTAINABLE BY VARIOUS TECHNIQUES

Readers unfamiliar with sterilization should note one general principle: if a homogeneous microbial population is exposed to a sterilizing agent under constant conditions, the population falls exponentially with time of exposure. Another way of stating this is: a plot of the logarithm of the population count vs. time is a straight

line. (Fig. 1. This is analogous to a radioactive decay curve.) Exponential kill curves are ordinarily found for exposure of microbes to heat, chemical agents, and radiation, but deviations sometimes occur in the direction of a slower fall-off after long time. Deviations are most commonly attributed to lack of homogeneity in the original population--that is, the presence of microbial strains with varying resistance to the sterilizing agent (Ref. 1, 2).

An exponential kill curve means that the time necessary for sterilization depends on the initial microbial count. Also, if an experimental kill curve is extrapolated to negative values of log population, these values may be interpreted as the probability that a single viable organism remains.

Sterilization by Heat

The sterilizing agent one thinks of first is moist heat, usually in a steam autoclave. Unfortunately, a great many spacecraft components will be damaged by heating in steam. More important, however, steam will not penetrate into sealed components, such as vacuum tubes, transistors, other electronic components, the interiors of many pieces of plastic, etc. Thus, these interiors are subjected only to dry heat, not to moist.

Dry heat may well be used for spacecraft sterilization. The temperature and time required for sterilization by dry heat are considerably greater than for moist heat. A dry heat cycle of 24 hr. at 135°C lowers the population of resistant bacterial spores by a factor of about 10^{-13} (Ref. 3, 4); no organisms more resistant to dry heat are known. Hobby has given an original count of 10^9 organisms on the surfaces and in the interior of the spacecraft as

representative of good clean practice (Ref. 6); the probability of a viable organism remaining after 24 hr. at 135°C is then about 10^{-4}.

With highly trained personnel, the use of temperature monitoring, and the supervision of experts on heat flow, the chance that some portion of a component or even of a complete spacecraft does not receive the intended heat cycle can probably be brought down to 10^{-6}. Many spacecraft components, especially electronic components and propellants, are, however, damaged even by 24 hr. at 135°C; components capable of withstanding this temperature can probably be developed, but time and money will be required to do so.

Sterilization by Radiation

Sterilization can also be accomplished by ultraviolet and by ionizing radiation. Ultraviolet radiation reaches only directly exposed surfaces, and is of no use for interiors, or for shadowed surfaces or holes. Ionizing radiation is more penetrating. Exposure to a dose of 5×10^6 rad has been reported to reduce a resistant microbial population to $10^{-5.3}$ of that originally present (Ref. 7). Thus, a dose of 10^7 rad of penetrating ionizing radiation should reduce the count by $10^{-10.6}$ and a dose of 1.2×10^7 rad should be enough to reduce the original count by a factor of 10^{-13}. Many plastics and elastomers, including solid propellants, as well as the optical properties of many glasses and pigments, will be damaged by 10^7 rad. It may be possible to develop components of adequate radiation resistance; time and money will again be needed.

Sterilization by Gas

Gas sterilization kills only microbes on exposed surfaces. It does not reach sealed interiors or, necessarily, interiors of closed screwholes, flanges, gasket seats, or electrical connectors.

Ethylene oxide is a good sterilizing gas and does not damage the great majority of spacecraft components. In appropriate concentration it has been shown to kill original populations of about 10^8 resistant organisms on paper patches in 11 hr exposure under appropriate temperature and humidity conditions (Ref. 8). If one wants a probability of 10^{-4} that a single viable organism remains, then starting with a surface population of 10^8 (Ref. 6), it is necessary to reduce the count by a factor of 10^{-12}. On the basis of an exponential kill curve, this would require about 17 hr for the conditions mentioned.

In using sterilizing gas, there are problems in making sure that the gas reaches all surfaces intended. There is also a chance of a human error or instrument error occurring: mistakes may be made in control of concentration, temperature, humidity, etc. There is estimated to be one chance in a thousand of a serious human error occurring. By having one human check on another, duplicating measuring instruments, etc. the probability of a breach in sterility arising from these sources could be reduced to less than 10^{-3}.

Some significant uncertainties remain, however, as to the effects of substrates (Ref. 9, 10, 11, 12) and of humidity and vacuum cycles (Ref. 2) different from those in the tests referred to. In particular, on some substrates, as well as with some presterilization humidity or vacuum cycles that may be of practical importance for spacecraft, there is evidence that ethylene oxide kill curves are not exponential but level out at 8 to 18 hr, so that longer exposure produces no further drop in population (Ref. 2, 11). It appears, therefore, that there is not yet sufficient evidence to show that ethylene oxide can be relied upon to produce high probabilities of sterility under

spacecraft conditions. Even fewer data are available for other gaseous sterilants of interest.

Sterilization by Liquids

In principle, it might be possible to dip components or a spacecraft or capsule in a sterilizing liquid; formaldehyde in methanol is one of the best (13). Just as with gas, the liquid will sterilize only the exposed surfaces which it touches. Moreover, because of the liquid's higher viscosity and surface tension, it will not reach many crevices that would be reached by a gaseous sterilant. For those surfaces that are reached, it is possible to get a low probability of contamination in a shorter time than is possible with gas. The contact times required are some hours, rather than a few minutes as sometimes thought (Ref. 14,13). Moreover, the effectiveness of liquid sterilants of interest seems rather erratic and apparently is sensitive to minor variations in sterilant concentration, quantity, evaporation, hydrolysis, polymerization, and storage time, as well as the substrate nature and cleanliness (Ref.13, 15). Experiments with liquid sterilants on simple spacecraft components previously inoculated with bacterial spores (Ref.15) showed that, at best, viable spores remained on the surface of 10^{-1} to 10^{-2} of the components used. For 100 components, the probability of contamination remaining would then be essentially unity.

Known liquid sterilants damage certain critical spacecraft components; in particular, they degrade the electrical properties of electrical connectors.

Internal Chemical Sterilization

Some materials used in spacecraft are inherently self-sterilizing; for example, fuming nitric acid, a propellant. For some

other materials and components,[a] sporicidal chemical may be added to the material in the course of its manufacture. This may be done in normal production or specifically for sterilization. The chance of contamination remaining depends upon the item and procedure used. To obtain quantitative assurance of the chance of contamination remaining, it will be necessary to obtain kill curves, for the particular chemical formulation and the conditions of use, which show the percent of original microbial populations remaining as a function of time. Alternatively, to know if the chance of contamination is 10^{-4}, it will be necessary to make over 10,000 tests, and find that not more than one of these show any contamination remaining. If addition of the internal chemical sterilant is not an indispensable part of the normal manufacturing procedure, then there remains a chance of mix-up or other human error leading to omission of the sterilant; the likelihood of such occurrences will be discussed later in this paper.

Sterilization by Filtration

For fluids, it is possible to use heat, radiation, and internal chemical sterilization, as already discussed. Sterilization by passing through a bacteriological filter may also be considered. For gases, fiber-type filters may be used. With a properly designed and properly prechecked and postchecked system, the chance of contamination with such filters can be brought to 10^{-3} or lower. Moreover, several filters could be used in series to increase the likelihood that no organisms get through. For liquids, fiber-type filters must be very thick, and provide very slow filtration. As an alternative, filters all of whose pores are smaller than the size of the organism may be used; with present technology, this means membrane

filters. Such filters are likely to fail because of surface scratching, fracture, or other imperfections. The assurance of sterilization is, therefore, only about 10^{-2}. Several such filters could be used in series to improve assurance. If these filters are of the same type, however, and particularly if they are from the same batch, there is considerable chance that all will show imperfections if any one does. Thus, the probability that all will fail may be greater than the product of the failure probabilities of the separate filters.

Aseptic Assembly

Aseptic procedures, similar to those used in surgery or bacteriology, have often been suggested for assembly of previously sterilized spacecraft components or subassemblies.

If the assembly is done in an ordinary fairly clean laboratory or electronics plant room, the probability of contamination from the air alone must be considered essentially unity per part. Air fallout, even under clean room conditions, contributes 10^{1} to 10^{3} organisms/ft^{2} hr that grow on bacterial culture media (Ref. 16,46,47,49) and perhaps a larger number of organisms for which these media are unsuitable. (It is clear that ordinary surgical procedures do not maintain sterility, but rather keep the number of pathogenic organisms introduced low enough for body defenses to handle.) If liquid sterilants are applied to mating surfaces during the assembly operation, the chance of contamination remaining is estimated to be 10^{-1} to 10^{-2}/part, or essentially unity for 100 parts.

A slightly better approach would be to assemble sterile bagged parts under a hood provided with a positive pressure of bacteriologically filtered air. The hood, sprayed in advance with a liquid sterilant, would be furnished with

ultraviolet lamps to further reduce contamination. Manipulations would be manual with the operator's hands in previously sterilized gloves. Under such circumstances, the chances of contamination from the bags, hands, gloves, and air are estimated at 10^{-2}/part. This estimate is based upon data that Food and Drug Administration inspectors in drug industry plants using this procedure picked up 55 infected lots out of about 1500 lots sampled in simple routine operations such as bottling of sterile injection solutions (Ref. 17). At 10^{-2}/part, the probability of contamination occurring in 100 parts would be about unity. If a liquid sterilant were applied to mating surfaces during the assembly operations in the hood, a reduction in the chance of contamination of 10^{-1} to 10^{-2}/part should be attainable, giving an over-all chance of contamination of 10^{-3} to 10^{-4}/part. For 100 parts this would amount to 10^{-2} or more.

If a hood assembly technique is used, but the parts brought in are externally infected, the assembly resulting would certainly contain trapped infection from the externally infected surfaces. Sterility would then depend on reaching microbes in crevices with a liquid sterilant; as mentioned above, then probability of contamination remaining would be near unity for 100 parts.

Sterile Assembly in a Glove Box

Perhaps the best technique of sterile assembly would be to assemble the parts in a glove box containing a sterilizing gas such as ethylene oxide. One difficulty is that no gloves are known to be sufficiently impervious to permit an operator to use them without severe blistering of his hands by the ethylene oxide which diffuses through the rubber or plastic. Present practices require a purge of the box

and gloves with sterile air for some hours after the ethylene oxide treatment before a man may insert his hands into the gloves (Ref. 12). With such a technique, if the parts to be assembled are internally sterile and are placed in the box before the ethylene oxide cycle, the reductions in contamination should be as described above for the ethylene oxide treatment. The chance of contaminated air leaking into the system after the ethylene oxide is removed must also be considered, however. If the purge air is properly filtered or heated to remove microbes and if the air system, the chamber itself, and the gloves have been properly pretested and monitored, the chance of such a leak could be reduced to 10^{-3} or lower. This estimate is based on the experience of the National Institutes of Health, where glove manipulations have been performed several times a week on several chambers containing germ-free mice for four years, with no break in sterility (Ref. 18). This record, setting a contamination level of 10^{-3} - 10^{-4} per manipulation or even lower, appears to be unusually good; it is based on use of permanent welded stainless steel boxes and highly trained personnel. With temporary or flexible enclosures, it would be more difficult to assure freedom from leaks and the chance of contamination would be higher.

A modification of this procedure would permit bringing internally sterile parts and tools into the sterilized glove box through an airlock. Leakage through the airlock closures increases the chance of contaminating the box. Normal indoor air admitted through the lock would contain 1 to 10 or more organisms/ft^3 (Ref. 16, 19, 46, 47, 48, 49); if this air were not sterilized, the chance of contamination would approach unity. Introduction of microbes on the exterior of the parts brought in, or on their containers,

means that there will be some chance of these microbes reaching supposedly sterile parts through contacts with box, gloves, and tools; the probability is estimated at 10^{-2}/part or higher; for 100 parts, the probability of contamination is essentially unity. Application of a liquid sterilant would reduce the probability of surface contamination by a factor of 10^{-1} to 10^{-2}/part, as mentioned previously. If sterile bagged parts are brought in though a U-tube containing liquid sterilant, the chance of the sterilant not reaching all organisms on the bags (especially at seals) is perhaps 10^{-3} to 10^{-4}/bag, or 10^{-1} to 10^{-2} for 100 bags. Exposure to the liquid for some hours would be required to reach this degree of assurance of sterilization.

Some plans for sterile assembly envisage transferring sterile fluids through piping and fittings into a presterilized spacecraft or capsule. The chance of contamination of piping and fittings is then important. It should be noted that techniques involving opening connections to laboratory air and applying a liquid sterilant to the surfaces leave 10^{-1} to 10^{-2} chance of contamination per part.

Many other variations of glove box technique could be suggested. It should be remembered, however, that a spacecraft is a complicated device; Ranger, for example, contained over 100 electrical connectors, 500 mating flanges, 1000 screws, etc. (Ref. 20). Assembly of a spacecraft is therefore a lengthy procedure, involving many people, and the size of the spacecraft makes glove techniques difficult (human arms may not be long enough to reach in from the box wall).

It should also be noted that if pieces are sterilized separately and then assembled, the probabilities of contamination must

be added over the total number of pieces involved, thus increasing the total probability of contamination. If the operating personnel and sterile procedures, or inspectors are not highly trained in sterilization techniques /the chance of a breach is very much increased, perhaps by a factor of 10^3.

Recontamination

With any of these methods of sterilization, the possibility of recontamination by subsequent leakage of nonsterile air into the container must be considered. In a welded leak-tested box, the chance of contamination through the seals is less than 10^{-6}. If the seal to keep out nonsterile air is less perfect, the chance will be greater.

In each stage of handling, there is some chance of contamination, the probability depend upon the design. Contamination of a previously sterilized spacecraft or capsule may occur on the pad, during transportation at the launching base, in the check-out areas, and during cross-country transport. It does not seem possible to attach numbers to these probabilities since they depend so heavily upon the design of the equipment.

If a sterile assembly operation is undertaken, there is a possibility of contamination of one of the subassemblies or components during handling prior to the sterile assembly. One method of handling items intended for subsequent sterile assembly is to sterilize and seal them in internally sterile bags, under the immediate control of a central sterility group, and thereafter never permit the bags out of custody of this group: that is, never permit the cognizant assembly

engineer and his technicians access for adjustment, modification, or repair. With this system of handling, the chance of mix-up in identification, other human error, or leak, may be a low as 10^{-6}/piece handled; for 100 pieces, this would amount to a total chance of contamination from these causes of 10^{-4}. If a double sign-off system is used at all stages, an additional reduction of 10^{-1} should be possible.

If the items are handled only by a central sterility group, but are allowed to be touched and externally contaminated, there is also a chance of contamination getting from the exterior to an interior area which is not reached by subsequent sterilization treatments. The probability would depend heavily upon the design and character of the item.

If sterilization takes place under the supervision of a spacecraft equipment vendor or cognizant assembly engineer and the package, sealed in an internally sterile bag, is then in the custody of the vendor or cognizant engineer, the chance of mix-up, other human error, or leak is estimated at 10^{-2}/piece; for 100 pieces the chance of contamination is then 10^{0}. With a double sign-off system, the chance could be reduced by a factor of 10^{-1}. (Note that for a spacecraft of the Mariner or Ranger class, there are typically about 30 to 50 cognizant engineers and hundreds of subassembly vendors).

When items are sterilized internally under supervision of a vendor or cognizant assembly engineer and are handled unsealed by him and his men, the chance of mix-up, tampering, or other human error is perhaps 10^{-1}/piece; for 100 pieces, loss of sterility is then virtually certain.

Effect of Launch Environment on Sterility

The possibility of a sterilized spacecraft becoming

contaminated during the launch must be also considered. This might occur, for example, by leakage into a closed shroud during the launch. The concentration of organisms in outdoor air varies strongly with season and meteorological conditions, but can be 10^3 organisms/ft^3 (volume at sea level pressure) or more; these are mostly fungal spores (Ref.19). [The bacterial count is about $10^1/ft^3$ (Ref.19, 21).] To attain contamination probabilities of 10^{-1} or 10^{-4}, the total volume of air permitted to leak into the shroud during the launch must then be less than 10^{-4} or 10^{-7} ft^3, respectively, during the entire period from sterilization to attainment of an altitude of 100 km. (It is generally assumed that Earth organisms do not go up to greater heights.) Since the air in the shroud must ordinarily flow out during ascent to permit pressure balance and removal of the shroud, this poses a difficult engineering problem in control of air flow.

It is also possible to contaminate a sterile spacecraft or capsule by a separation malfunction: by nonsterile materials staying with the sterile elements or touching them during separation. Such materials might include retrorocket gases or nozzle fragments, loose pieces or dust blown about by the gas or by the explosive separation devices which are often used, enclosure or barrier wrappings which do not separate properly, etc. The probability of this occurring depends entirely upon the design, particularly the design of the separation mechanism and procedure. It appears very difficult to get a really low probability of contamination during separation. There seems to be no way around the problem, however, except through sterilization in flight or on entry. Thus, excellent and failure-proof separation design is required for sterility.

The launch environment itself might be used to produce

sterilization. Aerodynamic heating during the launching is the only factor that seems applicable. If this were used, it might be possible to get a very low chance of contamination because the most likely source of abnormally low heating would be a low exit velocity, and if this occurred the spacecraft would not reach its target. To sterilize by aerodynamic heating during launch would, however, require a very special design of spacecraft and put extreme constraints upon the design. It is rather unlikely that this could be accepted.

Sterilization in Flight

Many people have asked whether the space environment itself would not be adequate to sterilize a spacecraft. There has been one report (Ref. 22) of sterilization by exposure to ultrahigh vacuum for 10 days or longer. On the other hand, a number of recent experiments (REF. 23, 24, 25, 50, 51) have shown that microbes remain viable after exposure to ultrahigh vacuum, even in tests up to 34 days. For the present, it must be said that no decrease in probability of contamination will result from vacuum exposure.

The outer surface of the spacecraft is exposed to solar ultraviolet and soft x-rays in those positions which face the Sun. This radiation will provide a good factor of kill on the exposed outer surface, but there are many regions of the spacecraft where the ultraviolet and soft X-rays do not reach. Therefore, the decrease in contamination probability because of solar ultraviolet and X-rays must be considered as zero.

Particle radiation is also present. If the spacecraft flies through the Van Allen radiation

belts, taking perhaps ten hours in transit, the dose received close to the surface, within a distance (shielding thickness) of 10^{-3} g/cm^2 or less, may be as much as 10^7 rad. This would be enough to sterilize to this small depth. Through the shielding thickness provided by many parts of the spacecraft structure (approximately 1 g/cm^2) the dose in ten hours would be less than 10^2 rad. This would kill no microbes.

Cosmic rays produce a total dose of the order of 10^0 to 10^1 rad/year even though heavy shielding; solar flare particles, according to present data, a total dose of 10^2 to 10^4 rad/year through 10^{-3} g/cm^2/ and less through heavier shielding. Such doses will not kill microbes.

Finally, sterilization by temperature encountered in space must be considered. There is a great deal of evidence that low temperatures, even in the liquid helium range, do not kill microorganisms. High temperatures will kill, but unless a heat sterilization cycle during the flight is deliberately designed into the spacecraft, most of the parts will not run above 100°C, and this is not hot enough to sterilize.

Sterilization in flight as a deliberate part of the flight sequence might be a useful technique. Chemical agents will not get into sealed interiors of spacecraft subassemblies or components nor can they easily be applied to the outside where high vacuum conditions exist. Heat sterilization during flight would work if the pertinent parts were kept hot enough for a long enough time. There is some chance, however, that the sterilization cycle will not operate as designed and that the mission nevertheless could not be aborted. It is a difficult engineering problem to heat a spacecraft evenly in space

and to avoid throwing instruments off calibration. If mechanisms are needed to provide sterilization in space, the reliability of the operation would probably not be better than 10^{-2}. In special cases, as when passive solar heating can be used directly to provide the temperatures needed, a lower chance of failure should be attainable. This technique has been suggested for sterilization of the last stage of a launch vehicle, after it has completed its operation and separated from the spacecraft (26). The probability of this scheme failing would depend primarily on the attitude conditions required with respect to the Sun and the method of obtaining and maintaining these conditions.

Sterilization and Release of Organisms During and After Entry

There have been suggestions that sterilization can be attained by ablation in a planetary atmosphere or by impact on a planetary surface. This requires some discussion.

If a heat shield is provided, sterilization by ablation is precluded. Even if no heat shield is provided, very small fragments, below 0.001 in. diam, will not reach temperatures high enough for sterilization. Such fragments might be blown or broken off the spacecraft during the early entry stages and would be analogous to the fine dust which enters a planetary atmosphere and reaches the surface without significant heating. Moreover, large pieces will not be heated sufficiently at their centers, during entry, to cause sterilization. Parts equivalent to polytetrafluoroethylene spheres larger than about 1/2-in. diam would not be sterilized by the heat of entry even at Venus, where entry heating is expected to be very severe (Ref. 27). This size is equivalent to a flat plate about 1/4 in. thick; planetary spacecraft will usually include a number of plastic parts of these

sizes, and entry heating therefore cannot be counted on to sterilize them.

Consider, next, sterilization from impact on the surface. If there is an atmosphere present, some fragments of the spacecraft will not hit the surface at high speed. Even if the spacecraft was deliberately designed to come in through the atmosphere very fast without break-up, such a design might fail, and it seems unlikely that a probability of better than 10^{-1} could be given for the chance that such a failure would not occur.

Suppose there is no atmosphere present, as on the Moon. There is good reason to believe that hitting the Moon at the hyperbolic velocity of 2 to 3 km/sec would not sterilize a vehicle. There are data (Ref.28) showing that some electronic devices can withstand impact accelerations of 200,000 g with relatively little damage; this is equivalent to a spacecraft impacting at 2 km/sec and being stopped within 1 meter. It seems certain that microbes aboard these pieces of equipment would likewise withstand the impact. Likewise, microbes survive chemical high explosions (Ref.29). Some decrease in the microbial count would probably take place.

If the organisms withstand entry or landing, what is the probability they will be released from the spacecraft or capsule to the planetary atmosphere or surface? From the outside of an entry body or lander, the probability is high, because the organisms may escape as dust during the initial stages of entry while still high in the atmosphere. From the inside of a spacecraft or capsule not designed to remain completely intact upon entry and landing, the probability of some release is again essentially unity. The number

released would be less than the total number of living organisms aboard, but the chance of contamination would not be reduced significantly unless the initial microbial population was very low indeed.

From the inside of a container designed to withstand entry and landing and remain completely intact, presumably no organisms would be released if the container performed exactly as intended. There is, however, always some chance of failure; it is possible that the container would break open. The chance of this is not likely to be less than 10^{-1} to 10^{-2}.

If only a very small number of organisms are released, we might obtain a factor of 10^{-1} to 10^{-2} that these do not include the varieties suited for growth under planetary conditions.

Avoiding Contamination by Missing the Planet

The easiest way to avoid contaminating a planet with Earth organisms is to avoid hitting the planet with spacecraft. We may classify our flights as entries, orbiters, and flybys. An entry flight is intended to hit a planet or its atmosphere; the chance of doing so is presumably somewhere near unity. For a planetary orbiter, in general, a rather small error or failure in the guidance or in the retro-propulsion will cause the spacecraft to enter the atmosphere of the planet. The chance of hitting the planet with an orbiter is therefore also high, probably near unity. For a flyby, the chance can be lowered as far as desired by aiming away from the planet; that is, increasing the planned miss-distance relative to the scatter in the guidance. This, however, lowers the value of the flight. An alternative is to introduce one or more midcourse maneuvers which lower the probable scatter in trajectory near the planet without

changing the planned miss-distance. Because a maneuver may not take place as intended [probability 5×10^{-1} to 1×10^{-2}, (Ref. 30),] the probability of hitting may not be lowered sufficiently unless the aiming point is initially set away from the planet ("biased"), and then reduced by the maneuvers.

Midcourse maneuvers add relatively little to weight. They add appreciable complication, however, and so lower mission reliability (Ref.31). Biasing an injection away from a target means that if a subsequent maneuver fails, the miss distance will be so great that the value of the shot will be considerably reduced as compared to a procedure in which no bias was used.

The probability of hitting the planet with the last stage of the launch vehicle should also be considered. If no avoidance or retro-maneuver is scheduled, the probability of this stage hitting the planet depends upon the injection errors. If a launch-vehicle stage avoidance or retro-maneuver is scheduled, then a factor of maneuver reliability must be introduced (10^{-1} to 10^{-2}). If impact probabilities are still not sufficiently low, one may consider going to a more reliable retro system, backing up the retro system with a second independent retro system, changing the planned injection trajectory, or sterilizing the launch vehicle. Retro systems on the launch vehicle add complications and again reduce reliability (32). Launch vehicle sterilization seems least practical, unless it can be done in flight, after cut-off (Ref.26). The reason is that launch vehicles have never been designed for unmanned planetary or lunar missions and perhaps never will be. They are designed for other purposes; persons responsible for scientific missions have no real

influence on of launch vehicles design/and cannot require that they must withstand sterilization.

THE ASSURANCE OF STERILITY REQUIRED

For Mars

On the basis of tests made under simulated Martian conditions, it appears that the chance of growth of some Earth organisms, if released on Mars, is essentially unity. There seems to be no question that we should attempt to avoid contaminating Mars with Earth organisms. Reasons have been outlined by the Cetex (Ref.33,34) and Westex Committees, by Davies and Comuntzis (Ref.35), Lederberg (Ref.36), Phillips and Hoffman (Ref.29), Hobby (Ref.6), and others. Basically, the objective is to permit biological observations of Mars without interference by organisms introduced from Earth. For engineering use it is necessary to prescribe, numerically, the degree of assurance that contamination will not occur; in other words, the risk of contamination that will be taken. (It is not possible to take zero risk, except by abandoning exploration of Mars). One way to obtain a reasonable number is to say that we should keep the chance of contamination of Mars as low as the chance we will obtain no useful biological data for other reasons. There are 7 oppositions of Mars remaining before 1980. If it is assumed that the USA and the USSR each attempt 2 flights to Mars at each opposition, about 28 flights may be tried. For each attempt, the probability of reaching the planet is perhaps 50%. If a spacecraft reaches the planet, it may still not return useful data on life because of failures in landing procedure, radio communication, power supply, scientific instrumentation or telemetry, unfortunate selection of landing site, etc. The probablity of such failure is

perhaps 50%. Thus, the probability that no useful data on life will be obtained in any one attempt is estimated as 3/4. The corresponding probability that no useful biological data will be obtained in the whole series of 28 attempts is $(3/4)^{28}$ or $10^{-3.5}$. One could then consider that the probability of contaminating Mars during the program should be kept as low as $10^{-3.5}$.

Another method of obtaining a working estimate is to say that the chance of contaminating Mars in the course of unmanned exploration should be kept low compared to the chance of contaminating it the first time a manned landing occurs. During a manned landing, contamination of Mars with Earth micro-organisms may occur through such factors as slight outward air leaks from spacesuits, the difficulty of reaching all crevices in space suits or other mechanisms by chemical surface sterilization, the added difficulty of sterilization in a spacecraft rather than in a laboratory, human error in Mars surface operations leading to a break in sterility, accidents on the Martian surface, crashes on landing, etc. Most engineers put the probability of microbes being released in this way as 10^{-1} or even higher. On this basis, 10^{-2} would be an adequately low number for the permissible probability of contamination during the unmanned program.

The numbers $10^{-3.5}$ and 10^{-2} for a program in which 14 flights reach Mars lead to $10^{-4.6}$ and $10^{-3.1}$ for the permissible probability of contamination on each flight. Perhaps an intermediate value of about 10^{-4} per flight is reasonable; Hobby (Ref.6) and the Space Science Board Study (Ref.37) have suggested this value.

It is obvious that reasons could be given for selecting widely different values; for example, 10^{-6} has been mentioned. The engineering difficulties of attaining even 10^{-4} are great; attaining

10^{-6} might require postponing unmanned exploration for many years.

Postponement of unmanned exploration may well reduce the number of unmanned attempts prior to manned landing and so reduce the chance of getting back meaningful biological data before contamination occurs.

For Venus

For Venus, the required degree of assurance against microbiological contamination can be modified by the chance that no environment suitable for growth would be encountered by Earth organisms on Venus. Measurements from Earth and from Mariner 2 (Ref. 38) indicate that the surface is too hot. There are, however, regions in the upper atmosphere of Venus which are suitable in temperature for Earth organisms. Earth micro-organisms apparently do not multiply in the atmosphere of Earth (Ref.19), and presumably would not do so in that of Venus. The chance of some Earth micro-organism finding suitable environment for growth on Venus is, therefore, estimated at 10^{-3}. Dividing the value of 10^{-4} per flight proposed for assurance against contamination, by this 10^{-3}, we obtain 10^{-1} per flight as the suggested assurance against releasing viable micro-organisms into the upper atmosphere of Venus.

For the Moon

For the Moon, sterility of spacecraft is probably not essential: it does not seem possible that Earth organisms could grow and reproduce on or near the surface of the Moon. Sagan (Ref.39).

and Imshenetsky (Ref.40) believe that there is a remote chance of growth, but no one else seems to share this view. The surface of the Moon, to a considerable depth, appears to be well below the freezing point of water, except for the outermost few centimeters which are exposed alternately to subzero and very high temperature, as well as to high vacuum and radiation.

The USSR has not published or released a description of the sterilization method used on Lunik II; it is therefore not known whether the spacecraft and launch vehicle, which hit the Moon, were sterile. Sterilization procedures were used on Ranger 4 spacecraft, which hit the Moon, but a few components probably were not sterile.

Lederberg and Cowie (Ref. 41) have pointed out that it is highly desirable not to contaminate the Moon to the extent that life scientists will be unable to determine whether organic substances found on the Moon are native or were brought there from Earth. The Moon has a surface area of 4×10^{17} cm^2 The average sample of lunar surface examined by an unmanned probe or even by an early manned explorer might be of the order of 10^3 cm^2. The chance of picking up an Earth organism within this area should be low compared to the other chances of contamination or error in a single experiment; let us say 10^{-3}. Accordingly, the probability of an Earth organism being found on the lunar surface should be held to $10^{-6}/cm^2$ surface. For the entire Moon, then, it would be undesirable to put down more than 4×10^{11} organisms from all unmanned flights. For a 40-flight unmanned program, this would mean less than 10^{10} organisms per flight, on the average. Ranger 4 is believed to have carried less than 10^7 viable organisms (Ref. 9). A bacterium weighs about 10^{-12}g; thus the total weight of viable organisms should preferably be limited to

10^{-2} g/flight.

Lederberg and Cowie (Ref. 41), Sagan (Ref.39), and others have pointed out that it is also undesirable to land dead organisms on the Moon. Unfortunately, there is no known way to remove these completely from a spacecraft. Decontamination procedures and cleanliness can lower their number.

Infection by Viruses

The sterilization schemes discussed above tacitly assume that only bacteria and larger organisms need be killed. Some of the techniques proposed, such as radiation and filtration, are less efficient for viruses than for other microbes, and may permit infectious viruses aboard spacecraft or capsules. This is probably justifiable on the basis that each virus can reproduce only in a specific type of living cell, and if Earth-type cells are excluded from a planet, Earth-type viruses could not reproduce there.

ASSOCIATED PROBLEMS

Monitoring and Accounting

Making sure that no breach in sterility has occurred will be a major undertaking unless such a breach is mechanically impossible. If sterile components or packages are to be handled, an organization is needed to monitor this handling and to keep careful records of which packages or parts have been sterilized. Manpower will be involved in this monitoring and accounting job. Sterility monitors will have to go along with each component to make sure that nothing is done to destroy its sterility; this responsibility could not be turned over to the cognizant assembly engineers without seriously degrading the likelihood of sterility. Checks are also needed to make sure that the monitors do not make mistakes.

Reliability of Spacecraft Functioning

Sterilization treatments of whatever sort may degrade reliability of operation. Preliminary results of an experimental study of sterilization by dry heat indicate that an appreciable increase in failure rate occurs in life testing of those electronic components not destroyed by the heat (Ref.42). There is also some evidence of increased failure rates during spacecraft checkout of electronic assemblies sterilized by dry heat (Ref. 20). This is most important, since there is little point in flying equipment which is likely to fail in service; reliability is usually the number one characteristic desired of a spacecraft. There are almost no data on the effects of sterilization techniques other than heat upon reliability. Data on reliability are not easy to obtain; many thousands of tests may be required.

Adjustments, Repairs and Recycles

The need for late calibrations and adjustment of equipment conflicts strongly with the sterility requirement. Current practices often involve manual access to the equipment and necessarily jeopardize sterility. The sterilization requirement suggests that equipment should be designed either to be self-calibrating or to be capable of remote calibration, with no manual access.

The need for late repair and replacement of components or subassemblies also conflicts seriously with sterilization procedures. This conflict can be reduced by placing sterilization as late in the sequence as possible so that repair and replacement can take place before rather than after sterilization.

If improper operation is found during a countdown it is often necessary to make corrections and repeat both the countdown

and a good deal of pre-countdown spacecraft preparation. The time required for such a recycle is always a problem for lunar and planetary shots. If re-sterilization is required, then the loss of time in the recycle may be very serious because of astronomical limitations on the launch period and firing window. Anything that interferes with 24-hr recycle times is highly undesirable. Sterilization procedures taking only a few minutes would be optimum.

Personnel Safety

Many of the procedures used for sterilization involve hazard to personnel. Beta-propiolactone is carcinogenic and is not currently recommended for use. Ethylene imine is also suspected of being carcinogenic. Both of these liquids blister skin. Ethylene oxide and formaldehyde are toxic in moderate concentrations. Ethylene oxide, even as commercially diluted, is inflammable when mixed with oxygen (Ref. 43). The alcohol solvents used with formaldehyde are inflammable. Heating may conceivably set off squibs and propellants. Radiation sterilization obviously involve some personnel hazards. Thus, certain precautions should be taken in sterilization procedures and certain risks to personnel accepted.

Training

Sterilization of such a complex mechanism as a spacecraft is in itself a complicated and difficult undertaking. Many of the problems involved do not show up until it has been tried. There is, therefore, considerable need for training of personnel in sterilization of spacecraft and perhaps of spacecraft components and for rehearsing the procedures that are to be used.

Costs

Sterilization costs money, time, manpower, and development effort. When these are short, sterilization is likely to be slighted or circumvented. Important psychologically is the very serious development effort needed on some components or subassemblies to make them compatible with sterilization requirements. Engineers responsible for the equipment may object strenuously on the grounds that they have enough headaches already. Their response is likely to be an attempt to find shortcut methods of sterilizing or methods of providing that sterilization is not really needed. There should also be realization that sterilization may compromise performance. For example, a propulsion system capable of sterilization to the required degree of assurance may be heavier than a similar system which need not be sterilized.

State-of-the-Art

Many techniques have been proposed for sterilization which are dependent upon the successful and timely completion of considerable research or advanced development. To be consistent with the philosophy of spacecraft design that has been successfully used in the past, such methods should be rejected for all missions already in the design or fabrication stage. Sterilization of spacecraft already in design or fabrication should be carried out by methods whose effectiveness and compatibility with the planned mission are already well established. If this is not done, mission completion or sterility will probably be seriously jeopardized.

DISCUSSION

Techniques for Lunar Landers and Orbiters

As mentioned above, it appears that sterility is not

needed for lunar flights. For lunar landers and orbiters, the mass of living material aboard should be held to about 10^{-2} g/flight. This level should be readily attainable with ordinary good techniques of spacecraft preparation and assembly.

Techniques for Venus Flybys and Orbiters

For Venus, it was suggested above that the probability of introducing a viable organisms into the atmosphere should be held to 10^{-1} per flight, for the present. This should generally be attainable on flybys without sterilization. With an intended miss distance of perhaps 30,000 km, injection-trajectory scatter typically gives an impact probability of about 10^{-2} for the spacecraft and the last stage of the launch vehicle (44,31). A midcourse maneuver, intended to reduce trajectory scatter near the target planet and so permit more accurate location of scientific instruments, will generally reduce the chance of unintended entry.

This "don't hit!" technique was used for Mariner 2. In addition, since the allowable impact probability was set lower when this flight was planned, a retro-maneuver was used for the last launch vehicle stage, and took place after this stage separated from the spacecraft.

It may also be practical to use the "don't hit!" technique for a Venus orbiter. The difficulty would be that large velocity changes are required to transfer a spacecraft from a Venus approach trajectory to the desired orbit , and a guidance or propulsion malfunction during this transfer could easily put the spacecraft into the atmosphere. Much care would be needed to keep the chance of this occuring below 10^{-1}. The chance of unintentionally achieving an orbit

that would decay into the atmosphere within a few years would also have to be held down.

Techniques for Venus Entry

Since the surface temperature of Venus is very high (Ref. 38), a lander, intended to operate on the surface, would almost certainly be designed to operate with high internal temperatures. Heat sterilization of the lander prior to launch, or perhaps in space, should therefore be a simple matter.

An entry capsule designed to return data from within the atmosphere but not to survive at the surface might not be so heat-resistant. It should not be too difficult, however, to devise a sterilization procedure which would hold to 10^{-1} the probability of contamination and prevent release of terrestial microbes into the upper atmosphere of Venus.

Techniques for Mars Flybys

For Mars missions, it was suggested above that the probability of introducing a viable organism to the planet should be held to 10^{-4}/flight. One possibility is to sterilize the spacecraft and last stage of the launch-vehicle; disadvantages of sterilization have been pointed out. An alternate approach would be to keep down the probability of entering the planetary atmosphere. For a Mars flyby, with an intended miss distance of perhaps 20,000 km, injection-trajectory scatter typically gives a probability of impact of about 10^{-2} (Ref. 45). This is not low enough for Mars. A midcourse maneuver would generally reduce the chance of unintended entry. The probability of midcourse maneuver failure is, however,

likely to be 10^{-1} or higher (Ref. 30,44), leaving the overall probability of entry above 10^{-3}. To reduce further the probability of entry, the injection trajectory can be aimed further away from the planet, and midcourse maneuvers used to reduce the miss distance to that desired for the mission. For example, biasing the injection trajectory to miss the planet by 50,000 km might be enough to lower the impact probability to 10^{-4}. Midcourse maneuvers could bring the trajectory back to 20,000 km from the target. If any of these maneuvers failed to occur, the entry probability would not be increased. Injection trajectory biasing would lower the probability of launch vehicle entry, just as it lowers the probability of spacecraft entry.

Techniques for Mars Orbiters

As was indicated above ("Venus Orbiters"), it seems difficult to hold to 10^{-1} the probability of a malfunction that would put an intended orbiter into the planetary atmosphere. To hold the probability of such a malfunction to 10^{-4} appears beyond the state-of-the-art. Accordingly, Mars orbiters should be sterilized, as outlined for Mars entry spacecraft, below. If the chance of entry can be held to 10^{-1}, then a probability of 10^{-3} could be allowed for a breach in sterility, and the overall probability of contamination still held to 10^{-4}. This might allow a slight relaxation of the procedures recommended for entry craft.

Techniques for Mars Entry

For a Mars entry capsule or lander, which is intended to "open" on Mars, the factor of 10^{-4} becomes the permissible likelihood that a viable organism is aboard. The technique that would give the lowest likelihood of a viable organism remaining is to heat the entire capsule or lander, including its propulsion system, in a sealed

container, insuring that all parts are held at a high enough temperature for an adequate time; say 135°C for 24 hr. Shorter times at higher temperatures can also be used. The inward leak rate of the sealed container during ground handling and launch would have to be extremely low, and the mechanism for separating lander from container such that the chance of contaminated materials touching or remaining with the lander during separation is lower than 10^{-4}. If any repairs or manual adjustment were needed on the sterilized lander, a complete resterilization should be carried out.

It is often asked whether sterile packages could not be added to a previously sterilized lander using sterile assembly techniques. The assembly technique giving the best chance of sterility is to use a closed glove box. Sterile parts, tools, and lander, sealed in internally sterile plastic bags, would be placed inside and the box and bags sterilized with ethylene oxide (for, say, 17 hr) and purged with sterile air or nitrogen before proceeding with glove assembly. Liquid sterilants would not be necessary. Additional tests of the adequacy of the ethylene oxide treatment are needed. To eliminate air leaks into the box with the required degree of assurance, very careful design, fabrication, and monitoring would be required. The packages or parts to be added would have to be previously sterilized by heat or radiation, or assembled by sterile glove box techniques from components and materials sterilized by these same techniques or by internal chemical sporicides of still-to-be established effectiveness. All sterilization and sterile assembly would have to be under the direct control of a central sterility group. The cognizant assembly engineer could have no access to his sterilized

equipment, components, or materials for testing, repair, modification, assembly, or fabrication, except for sterile glove-box operations under direct control of the sterility group. A rather elaborate system of accounting for and monitoring all sterile items would be necessary. Even with all these precautions, it is not really certain that glove-box assembly can provide as low as 10^{-4} probability of contamination; the technique can at best be considered a poor second-choice to overall sterilization by heat.

The possibility of carrying out a sterile assembly without a glove box by using liquid sterilants has been considered. In an open room, surfaces would rapidly become contaminated and liquid sterilants will not provide 10^{-4} assurance of sterility.

Sterile gases might be added to a lander through an appropriate filtering or heating system. In principle, liquids might also be added in these ways or, if the liquids are themselves of such chemical nature as to be adequately sporicidal, in simpler ways. If dependence is placed on filtration of liquids, a series of filters would have to be used to increase assurance of sterilization. Filters of reasonable speed have marginal reliability for attaining assurances as great as 10^{-4} and extreme precautions would have to be taken to minimize the chance that several of the filters used in series are defective. With either liquids or gases, plumbing connections to a sterile lander should be made only to the nonsterile side of a sterilization barrier (filter, heater, or pipe full of sporicidal liquid). A suitable separation design would be needed to remove nonsterile parts of the plumbing from the spacecraft without exposing the latter to possible contamination. This may imply "remote" separation, with its additional complications, rather than "manual" separation

of the plumbing.

It is perhaps worth repeating that many methods of obtaining sterility which are commonly used and widely advocated will not provide the necessary degree of assurance of sterility for a planetary mission.

CONCLUSIONS

1. An entry capsule or lander for Mars should be sterilized and handled with procedures assuring that the probability of a single viable organism being aboard is not over 10^{-4}. This figure takes into account the probabilities of sterilization during Mars entry and impact and of releasing organisms from the capsule at the planet.
2. To achieve this probability, either the lander should be sterilized in space, or sealing and separation mechanisms must be designed to provide a very high degree of assurance that leaks and malfunctions which would contaminate the lander cannot occur prior to or during launch or at separation of the lander from shroud and launch vehicle.
3. Sterilization of a Mars lander should, if at all possible, be by heat, either in space or in the final sealed container, with no access permitted or mechanically possible thereafter, except with complete resterilization by heat. A considerable amount of development work on spacecraft and spacecraft components will be needed before this becomes feasible.
4. If heat sterilization of the complete lander is impossible, then, as a rather poor second choice, heat sterilization should be used on as large a lander assembly as possible, and sterile parts, including fluids, added by a glove box procedure using ethylene

oxide in the box. All packages, components, materials, fluids, and tools used should have been previously sterilized under the direct control of a central sterility group, sealed in internally sterile containers, and retained at all times in the possession of the central sterility group. Cognizant assembly engineers should not be allowed access to sterile packages, components, materials, or fluids for testing, repair, adjustment, calibration, assembly, or fabrication, except for glove box operations under direction control of the central sterility group. A rigid system of accounting and monitoring should be established and enforced. A considerable amount of work will be needed to establish the adequacy of this procedure.

5. For Mars orbiters, sterilization should also be required. The probability of a breach in sterility and the probability of unintentionally hitting the atmosphere, as by a malfunction, should be controlled so as to hold the overall probability of introducing a viable Earth organism into the atmosphere of Mars to 10^{-4}.

6. For Mars and Venus flyby spacecraft, for Venus orbiters, and for the last stages of launch vehicles, either sterilization should be used, or trajectories should be controlled to ensure not over 10^{-4} probability of hitting Mars and 10^{-1} probability of hitting Venus. For Venus entry craft, sterilization should continue to be required until further data are obtained.

7. For lunar missions, sterilization does not seem essential. Cleanliness procedures should be used to assure that no more than

0.01 g/flight of living matter is deposited on the Moon.

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